A STUDY OF THE RESPIRATORY MECHANISMS FUNCTIONAL IN THE EARLY EMBRYOLOGY OF THE FROG, *RANA PIPIENS*

BY

MARTHA REVEL BARNES
A.B., University of Illinois, 1938
M.S., University of Illinois, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY
IN THE GRADUATE SCHOOL OF THE UNIVERSITY
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INTRODUCTION

The following work represents a study of the metabolism of the developing amphibian, *Rana pipiens*, as revealed by measurement of oxygen uptake, use of specific inhibitors, and addition of the substrate, glucose, to enzyme extracts from the embryonic tissues. It was first suggested by an examination of the distinct morphological changes occurring in development with which it was hoped physiological activities could be correlated.

Different developmental stages may be associated with different cellular activities. The period of cleavage is characterized by cell divisions. Gastrulation is accomplished largely by cell movements. Neurulation marks the very beginning of cell differentiation. Certainly these phases overlap, but in general a distinct serial relationship holds.

The question now arises as to whether these different cellular activities can be correlated with distinct and peculiar metabolic systems. It was hoped that if metabolic differences could be established, some clue as to the actual cause of the changes in cellular activities could be gained. Therefore, it was decided that a consideration of the possible energy-release systems and the procuring of experimental evidence for the existence of such systems might offer valuable information to a general understanding of the physiology of the amphibian embryo.

A Warburg apparatus was used for the oxygen determinations. Potassium and sodium cyanide, ethyl urethane, and monoiodoacetic acid were employed as the inhibitors. The embryos were staged according to Shumway's normal tables.

RESULTS

The following results were obtained.

1. Oxygen consumption in embryos of *Rana pipiens* increases exponentially with age from fertilization to the 3-mm stage with a marked discontinuity during the formation of the neural folds. These results check with those obtained by Atlas (1937) using the same animal. The constant of the exponential equation which fits the curve changes at the time of neural-fold formation.

2. The blastula stage is more sensitive to ethyl urethane than any of the earlier stages. Following the blastula stage, narcotic sensitivity decreases with age. Development in the vegetal region is more greatly inhibited than it is in any other portion of the embryo. Urethane, 2%, inhibits 43% of the respiration of early cleavage stages, 57% of that of the blastula, and 34% of that of the gastrula.

3. Cleavage proceeds normally up to the blastula stage in M/1000 cyanide but gastrulation cannot occur in the cyanide solution. If however, the embryo is exposed after formation of the dorsal lip it will proceed to the mid-gastrula stage. Neural-fold formation cannot occur in cyanide. The maximum inhibition of the respiration in early cleavage is 53%; that in the blastula is 71%; and that in the gastrula is 88%.

4. Development up to the stage of the neurula proceeds normally in the presence of monoiodoacetic acid, M/000, but the formation of the neural ridges is completely inhibited. The respiration is unaffected in all stages except in the neurula where it is inhibited 40%. This inhibition is explainable by a decrease in the production of the substrate glucose.

5. Glucose added to enzyme extracts accelerates oxygen consumption up to the mid-gastrula stage; it inhibits the respiration of late gastrulae and neurulae.

DISCUSSION

From the inhibitors studied, it is suggested that distinct kinds of cell activity can be correlated with different energy-release systems. Cell division in amphibian embryos can proceed by a system which is cyanide-insensitive, monoiodoacetic-acid-insensitive, and relatively narcotic-sensitive. Such a system is to be found among those systems, which in the presence of a dehydrogenase, allows the hydrogen from the substrate to combine directly with atmospheric oxygen. Gastrulation, involving cell migration, is characterized by a sensitivity to cyanide. This suggests that oxidations are needed which are produced in the presence of an enzyme containing a heavy metal as an active group. Differentiation in amphibian development, as displayed in neurulation, is distinguished by its sensitivity to all inhibitors employed in these experiments. Its peculiar sensitivity to monoiodoacetic acid would indicate that glycolysis is necessary to the beginning of the differentiation of the nervous

system. The over-lapping of developmental activities and the energy-release systems correlated with them is demonstrated by the sensitivity of the blastula to narcotic only, of the gastrula to narcotic and cyanide, and of the neurula to narcotic, cyanide, and monoiodoacetic acid.

The increase in rate of oxygen consumption can be correlated with the addition of living embryonic tissue. The cause of the break in rate of oxygen usage at the appearance of the neural ridges may be due, as suggested by Atlas to the first increase in body length, or as suggested by Philips to the cessation of cell division during periods of movement and differentiation, or as a third alternative, to the appearance of glycolysis as an energy-yielding mechanism.

Since glucose added to an extract prepared from cleaving eggs and young gastrulae stimulated oxygen uptake, it is believed that the enzymes for sugar oxidations are present in these early stages but that the lack of substrates prevents their activity. The inhibition of similar enzyme extracts from late gastrulae and neurulae is perhaps due to an accumulation of toxic intermediaries or to a reversal of the synthesis reaction due to the presence of an excess of end product. These facts can be correlated with the work of Brachet, (1934), on respiratory quotients and glycolysis determinations.

CONCLUSIONS

It is concluded that respiratory mechanisms other than those involving the use of atmospheric oxygen can supply the needs of the embryo during cleavage up to the blastula stage, but that oxidation mechanisms are necessary to complete gastrulation and that glycolysis is required for formation of the neural folds.

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VITA

Martha Revel Barnes was born in Springfield, Illinois, October 6th, 1915. After completing her elementary schooling in a public school of that city, in 1929, she entered Bowen High School in Chicago, Illinois, graduating in 1933. She then entered the University of Illinois in Urbana. She secured her Bachelor of Arts degree from this university in 1938 with a major in zoology and in 1939 she received a Master of Science degree in the same subject.

At the university she held a graduate assistantship in Zoology during the years 1939 to 1941 including the summer of 1941. She spent the summers of 1939 and 1940 at the Marine Biological Laboratory at Woods Hole, Massachusetts. The last summer of her work was made possible by a grant from the University of Illinois.

In 1940 she was received as an active member into Sigma Xi.

Her publications are as follows:

Barnes, Martha Revel (1939) Glycogenetic activity in early stages of the developing amphibian. Anat. Rec., 75:114-115.

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